# Correlation of HbA1c with atherogenic index of plasma and endothelial dysfunction in type 2 diabetic subjects

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## Abstract
**Introduction:** Endothelial dysfunction (ED) is a well known initial stage of atherosclerosis and cardiovascular disease (CVD) in general population. Recently studies shown increased HbA1c is a marker for cardiovascular risk in subjects with diabetes and without diabetes. Atherogenic index of plasma (AIP) is another risk factor for cardiovascular events. Hence, the study has been designed to assess the correlation of HbA1c with AIP and ED in type-2 diabetic subjects to forecast risk of cardiovascular events.
**Materials and Methods:** Hundred subjects are enrolled into study with the age of 38 to 58 years, among these 50 were type 2 diabetic subjects and 50 were healthy controls. HbA1c was estimated by immuno-turbidimetric method. Nitric oxide (NO) was measured by kinetic cadmium reduction method and AIP was calculated by standard formula Log (triglyceride/HDL-c). 'Kruskal Wallis' test was used to perform statistical variables between the study groups. The association between the variables was executed by "Pearson correlation test".
**Result:** HbA1c level was shown to be significantly higher in type-2 diabetic subjects than healthy controls. Significant lower level of NO was observed in type-2 diabetic subjects than healthy controls. AIP level was significantly higher in type-2 diabetic subjects than healthy controls. The present study also observed significant correlation of HbA1c with NO and AIP.
**Conclusion:** We have found that HbA1c has significant correlation with NO and AIP. Therefore, estimation of HbA1c may be a predictable marker for cardiovascular events in type-2 diabetic subjects.

**Keywords:** Glycated hemoglobin, Atherogenic index of plasma, Nitric oxide, Cardiovascular disease.

## Introduction
HbA1c is a non-enzymatic reaction of glucose and hemoglobin. Increased HbA1c level indicates uncontrolled glycemic status in diabetes mellitus and also acts as marker for cardiovascular risk. HbA1c measurement is an assay to monitor for long-term glycemic control in diabetes mellitus. It is an average blood glucose level during the period of 60-90 days. It has to be check at least twice in a year, because HbA1c concentration has direct association with diabetic microvascular complications.[1] HbA1c was also associated with severity of coronary atherosclerosis in men undergoing coronary angiography.[2] Another study revealed that risk of coronary heart disease (CHD) with diabetes is high in women than men.[3] However, Selvin et al reported that there was no association between HbA1c and prevalence of CVD in diabetic subjects despite an association with carotid intimal thickness.[4]

Dyslipidaemia is a major atherogenic factor to cause cardiovascular events in diabetic subjects. Atherogenic index of plasma (AIP) is a logarithmically transformed ratio of molar concentration of triglyceride to HDL-cholesterol. It is strongly correlated with lipoprotein particles size and various indexes have been used to diagnosis and prognosis of cardiovascular disease.[5]

However, endothelial dysfunction is a well known primary cause of atherosclerosis and cardiovascular disease in all populations. In normal condition, endothelium maintains normal vascular tone, platelet activity, leukocyte adhesion and thrombosis. Impaired endothelial function initiates the pathogenesis of atherosclerosis.[6,7] Nitric oxide is the most effective endogenous vasodilator of endothelial function. Reduced availability of NO causes impaired vasodilatation leads to endothelial dysfunction. It is a strong interpreter of cardiovascular event in patients with diabetes mellitus.[7]

Various studies have shown HbA1c is a risk factor for cardiovascular disease with and without diabetes, but still it is inconsistent. However, Nitric oxide and AIP are more predictable markers for pathological process of atherosclerosis and cardiovascular disease. Hence, the study has been designed to assess the correlation of HbA1c with AIP and endothelial dysfunction in diabetic subjects without any complications.

## Materials and Methods
Hundred subjects were enrolled in the present study. Among them 50 were type 2 diabetic subjects and remaining 50 were healthy controls with the age of 38-60 years.

The subjects were recruited into the study from OPD at Govt. Hospital and Vinayaka Mission Kirupananda Variyar (VMKV) Medical College, Salem, Tamil Nadu.
**Ethical Clearance:** The study was approved by the institutional ethical committee from VMKV Medical

College at Salem. Informed consent was obtained before blood sample collection from each subject.

**Sample Collection:** 4 ml venous blood sample was collected after an overnight fast of 12 hours. 1 ml blood sample was transferred to fluoride tube for blood glucose, 1 ml of sample transferred to plane tube for lipid profile, 1 ml of sample transferred to EDTA tube for HbA1c and 1 ml of blood sample transferred to heparin tube for NO.

**Methods:** FBS, PPBS were estimated by glucose oxidase peroxidase (GOD-POD) method. HbA1c was estimated by turbidimetric immunoassay method. Total cholesterol was measured by cholesterol esterase and peroxidase. Triglyceride was estimated by glycerol phosphate oxidase and peroxidase. HDL was measured by immune-inhibition, 2 reagent method. LDL-C and VLDL-C were calculated by using Friedewald's formula. Nitric oxide (NO) was measured by kinetic cadmium reduction method[8] and AIP was calculated by using standard formula Log (triglyceride/HDL-c).[9] Body Mass Index (BMI) was calculated by using the formula = weight (kg)/height$^2$ (meters).[10]

**Statistical Analysis**

Data analysis was done by using SPSS software version 20; Non-Parametric, "Kruskal Wallis" test was used to analyze statistical significance of variables between the two groups. p value <0.05 was considered as significant. Association of HbA1c with NO and AIP was performed by using "Pearson Correlation" test.

**Result**

Clinical and biochemical characteristics of the study subjects are shown in Table/Fig. 1. Significant high levels of BMI, Fasting and postprandial blood glucose were observed in type-2 diabetic subjects (24.30±3.04; 159.85±68.49; 277.98±85.90) compared to healthy controls (20.42±0.84; 87.94±10.14; 119.60±6.23), 'p' value <0.05 [Table 1]. But, there was no significant difference in the age among the study groups.

HbA1c was found to be significantly high in type-2 diabetic subjects (9.11±2.42) than healthy controls (5.21±0.30), p value <0.05 [Fig. 1].

[Table 2] shows significantly higher levels of total cholesterol, triglyceride, LDL and VLDL in type-2 diabetic subjects (200.90±38.96; 167.04±65.90; 192.52±40.06; 33.40±13.26) than healthy controls (158.34±22.03; 102.06±31.94; 97.37±25.15 & 20.41±6.39), p value <0.05. But, there was no significant difference in the level of HDL among the groups.

Significantly lower level of NO was found in type-2 diabetic subjects (13.76±8.01) compared to healthy controls (18.51±9.47), 'p' value at <0.05 [Fig. 3].

HbA1c was significant correlation with NO and AIP. HbA1c positively correlated with AIP and negatively correlated with NO [Table 3].

**Table 1: Clinical and biochemical characteristics of study subjects**

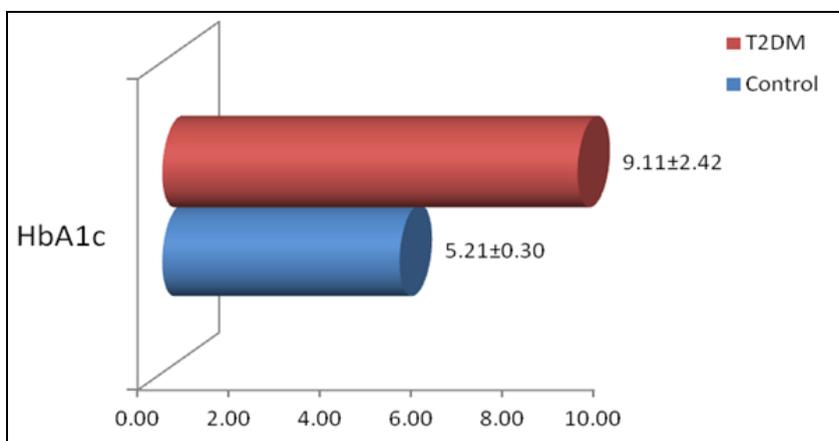| Parameters | Healthy Controls (n=50) Mean± SD | T2DM (n=50) Mean± SD | 'p' Value |
|---|---|---|---|
| Age (years) | 48.36±10.61 | 50.88±10.10 | >0.05 |
| BMI (Kg/m2) | 20.42±0.84 | 24.30±3.04 | <0.05* |
| FBS (mg/dl) | 87.94±10.14 | 159.85±68.49 | <0.05* |
| PPBS (mg/dl) | 119.60±6.23 | 277.98±85.90 | <0.05* |

*Significant at p value <0.05.



**Fig. 1: Level of HbA1c among study subjects**

**Table 2: Study the difference in the level of lipid profile among the groups**

| Parameters | Healthy Controls (n=50) Mean± SD | T2DM (n=50) Mean± SD | 'p' Value |
|---|---|---|---|
| T. Cholesterol (mg/dl) | 158.34±22.03 | 200.90±38.96 | <0.05* |
| Triglyceride (mg/dl) | 102.06±31.94 | 167.04±65.90 | <0.05* |
| HDL (mg/dl) | 40.56±9.14 | 41.48±8.20 | >0.05 |
| LDL (mg/dl) | 97.37±25.15 | 192.52±40.06 | <0.05* |
| VLDL (mg/dl) | 20.41±6.39 | 33.40±13.26 | <0.05* |

*Significant at p value <0.05.
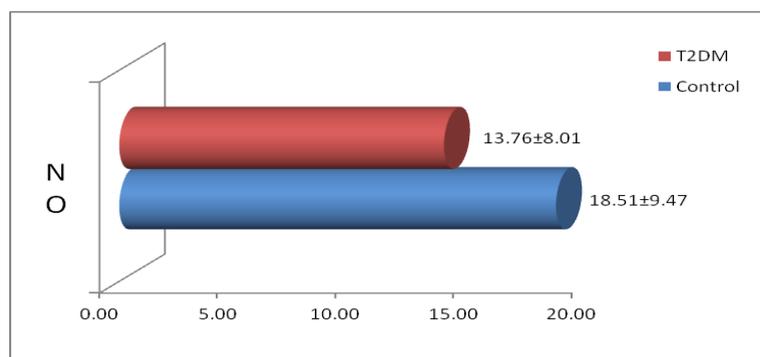


**Fig. 2: Level of nitric oxide between T2DM and control**

**Table 3: Correlation of HbA1c with endothelial dysfunction and AIP**

| Correlation of HbA1c | | |
|---|---|---|
| Parameter | 'r' Value | 'p' Value |
| NO | -236 | 0.015 |
| AIP | 346 | 0.000 |

AIP was positively correlated with HbA1c

NO was negatively correlated with HbA1c

## Discussion

Cardiovascular disease is the main cause of increased mortality among type-1 and type-2 diabetic subjects.[11] Diabetic complications can be reduced by controlling triglyceride levels. Treatment with insulin or oral hypoglycemic drugs delays the onset and slows down the progression of diabetic microvascular complications in type-1 and type-2 diabetes, but macrovacular complications is still uncertain.[12]

Prevalence of vascular complications was found to be high in type-2 diabetic mellitus. The present study type-2 diabetic subjects are uncontrolled in condition with higher level of fasting and postprandial glucose. Hyperglycaemia appears to be the significant key factor for development of diabetic complications.[13] It also shown significantly high levels of BMI in subjects with type-2 diabetes compared to healthy control. Ganz, M. L et al has reported that type-2 diabetic subjects have higher level of BMI than control.[14] Hollander P, revealed that weight gain in type-2 diabetes is mainly due to the excess of calorie intake, reduced glycosuria and physical inactivity.[15] Weight gain is a well-known factor for adverse effects of cardiovascular risk. Extreme weight gain declines the glycemic control and increases cardiovascular disease.[16]

**Glycated Hemoglobin (HbA1c):** The present study has shown significantly higher level of HbA1c in type 2 diabetic subjects than healthy controls. It was known that HbA1c levels were significantly high in in Type-1 and type-2 diabetes compared to non-diabetic subjects.[17] Zounga S et al 2012 has observed non-linear relationship between mean HbA1c during follow-up and risks of macrovascular, microvascular events and death. It has also reported that every 1% increase in HbA1c level was associated with 38% higher risk of macrovascular events, 40% higher risk of microvascular events and 38% higher risk of death.[18] UK Prospective Diabetes Study demonstrated that strict glycemic control can reduce the risk of microvascular disease.[19] Glycemic control also had shown favorable effects on cardiovascular disease in diabetic subjects.[20] A significant correlation has noted between HbA1c and lipid abnormality and it was also suggested for the importance of control of diabetes and lipid control. Focusing on HbA1c and lipid control can prevent the morbidity and mortality in diabetes.[21,22] In our study we have found significantly higher level of HbA1c in type 2 diabetic subjects due to poor glycemic control.

**Lipid Profile:** Dyslipidemia is an established risk factor for coronary heart disease (CHD) in both diabetic and non-diabetic subjects.[23] In our study, significantly higher level of total cholesterol, triglyceride, LDL and VLDL were observed in type-2 diabetic subjects than healthy controls. Mullugeta Y et al had reported elevated level of total cholesterol, triglyceride and LDL in poor glycemic control diabetic subjects than good glycemic control diabetic subjects.[24] Mohammadi et al reported elevated total lipid, triglyceride and LDL-c levels in poor glycemic control diabetic subjects (HbA1c->8%) than healthy controls and also he

confirmed the direct association of glycemic control and dyslipidemia.[25] Reduced activity of lipoprotein lipase (LPL) causes elevation of triglycerides level in hyperglycemic subjects. LPL hydrolyzes triglycerides of chylomicrons and very low density lipoproteins. The free fatty acid triggers triglyceride synthesis later in turn stimulates the synthesis of VLDL-c.[26,27] Khan reported that HbA1c was associated with cholesterol, triglyceride and LDL-c by analyzing laboratory data from 2200 type-2 diabetic subjects.[28] In our study, poor glycemic control might be the reason for elevation of cholesterol, triglyceride and LDL-c in type-2 diabetic subjects.

**Atherogenic Index of Plasma (AIP):** It is a logarithmically transformed ratio of molar concentration of triglyceride to HDL cholesterol. The present study observed significantly high level of AIP in type-2 diabetic subjects than healthy controls. However, decreased AIP was observed in type-2 diabetic subjects who were treated with hypoglycemic drugs with combination of lipid lowering drugs (ciplofaxin and combination of statin & niacin). It is also revealed that AIP is a sensitive marker of differences the lipoprotein profile families in patients with premature myocardial infarction and healthy controls.[5] An earlier study has reported remarkable effect of smoking, physical exercise, blood pressure and fasting blood sugar on AIP. It also reported strong correlation between BMI, total cholesterol and AIP in cardiovascular subjects.[29] In our study, poor glycemic control and dyslipidemia might be the reason for elevated AIP level in type-2 diabetic subjects.

**Endothelial Dysfunction:** Vascular endothelium plays a crucial role in vascular homeostasis. Insulin resistance is an important factor for CVD, a reciprocal relationship with endothelial dysfunction.[30] Naturally, type-2 diabetes is a state of insulin resistance, hyperglycemia and dyslipidemia, which accelerates atherosclerosis and leads to cardiovascular risk.[31] Reduced bioavailability of NO is the indication for endothelial dysfunction. In our study, we have found significantly lower level of NO in type-2 diabetic subjects compared to healthy control, this is supported by Versari D et al. 2009.[32] Hyperglycemia contributes to endothelial dysfunction in diabetes by several mechanisms. Limited availability of NADPH, a significant co-factor for eNOS (endothelial nitric oxide synthase), may occur due to reduced pentose phosphate pathway and leads to decrease NO production.[31] In other way, elevated LDL-cholesterol is susceptible to oxidation and produces oxidized LPL-cholesterol. This increases the synthesis of caveolin-1, which inactivates eNOS and reduces NO production.[33] In our study, poor glycemic control and elevated LDL-c might be the reason for endothelial dysfunction in type-2 diabetic subjects.

## Correlation

Evidently AIP and NO are significant factors to predict cardiovascular risk in general population and type-2 diabetic subjects. Hence, the present study was done, correlation among HbA1c, AIP and NO. The study was found HbA1c has significantly correlated with AIP and NO. AIP was positively correlated with HbA1c and NO was negatively correlated. However, an earlier study has found that mean glycemia and HbA1c has stronger association with CVD risk factors than fasting and postprandial glucose level in diabetic subjects.[34] Recent studies have been identified elevated level of HbA1c was associated with cardiovascular disease in general population without diabetes.[35] Hence, HbA1c may be independent predictable marker for cardiovascular risk in subjects with type-2 diabetes mellitus.

## Limitations

The present study was focused only on association of HbA1c with AIP and NO. Large studies are required to evaluate the correlation of HbA1c with NO and AIP based on different glycemic control status in type-2 diabetic subjects. This might provide comprehensible picture on whether HbA1c can be used as independent predictable marker for cardiovascular risk.

## Conclusion

We have found significantly higher level of HbA1c and AIP, significantly lower level of NO in type-2 diabetic subjects. We also stated HbA1c has significant correlation with AIP and NO. Hence, estimation of HbA1c may be a predictable marker for cardiovascular events in type-2 diabetic subjects.

**Conflict of Interest:** No conflict of interest

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